RESEARCH ON CUTTING PROPAGATION OF *GARDENIA JASMINOIDES*

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SUMMARY

The article introduces the results of the research on clonal propagation by cuttings method of *Gardenia jasminoides,* using type hormones and different concentrations obtained results with rooting ratio; while average roots per cuttings; average root length per cuttings and rooting index; total number of shoots; average shoots per cuttings; and average shoots length per cuttings are very different, when using Indole-3-butyric acid (IBA) achieved higher results. The usage of IBA at concentration of 1500 ppm obtained the results with the highest rooting rate (83.4%), the average number of roots per cuttings was 29.9 roots, average root length per cuttings was 3.3 cm, and the rooting index achieved was 97.9. Using α-Naphthalene acetic acid (NAA) at concentrations of 250 and 500 ppm (CT5 and CT6) obtained results with the highest rooting ratio, and total sproutings shooting (80.0% and 126 shoots respectively), while at higher concentration (1500 ppm) obtatined results reaching the highest value with the average number of roots per cuttings, and rooting index are 16.9 and 59.7 respectively. Using IBA (CT2) and NAA (CT6) at concentrations of 500 ppm in order to propagate Gardenia jasminoides by cuttings was able to promote sprouting shooting with highest values achieved were 129 and 126 respectively. The results of this study are the basis for further studies to get the success of the clonal propagation of *G. jasminoides* for planting and development.

Keywords: Cuttings, IBA, NAA, propagation of *G. jasminoides*, rooting ratio.

1. INTRODUCTION

The science name of Danh danh is *Gardenia jasminoides* Ellis which belongs to Genus (*Gardenia*), Family (*Rubiaceae*). The other science names of it are *Gardenia florida* L., *Gardenia angustifolia* Lodd, and *Gardenia augusta* L which is a small tree with ecologically quite wide and multi-effects. In addition, there are also other names such as Chi tu, Tien tu, Son chi tu, Luc chi tu, Hong chi tu, Hoang chi tu, Thuy hoang chi, Moc ban, Viet dao, Tru dao, Viet dong, and Mac lang cuong (Tay ethnic people) (Huy H. Le, 2016; Loi T. Do, 2007; Tuan D. Pham, Tho V. Hoang, 2008).

Gardeniajasminoides is a native species, forevergreen trees, the trunks reaching 1 to 2 m in height, many branches, leaves grow opposite or round 3; the flowers are usually white or ivory white, very fragrant and beautiful, so they are often used as decorative plants in many families from urban to rural area in Vietnam. Beside the value of ornamental plants, recent research shows that other parts of plants such as leaves, roots and fruits of these trees can be used as pharmaceutical raw materials for the treatment of diseases such as acute nephritis; urination, urinary stones; kidney stones; jaundice, yellowing of eyes, hepatitis; throat ulcer; headache, tinnitus, nosebleeds; hemorrhage, coughing up blood; epistaxis; sprain, aches; and especially, the preservation is the main ingredient used to make "kidney-beneficial" supplements with enhanced antioxidants, diuretics, kidney protection, as well as symptom relief and complications renal impairment such as edema, frequent urination, elevated creatinine or proteinuria is very effective and preferred by people (Huy H. Le, 2016; Loi T. Do, 2007; Tuan D. Pham, Tho V. Hoang, 2008).

At present, the demand for planting *Gardeniajasminoides* to create pharmaceutical raw material areas are particularly interested by many pharmaceutical companies and people. However, the thing of using seed for planting to achieve the desired results often fails because the seeds only have ratio low germination. Furthermore, collecting a large amount of seeds for production of seedling is very difficult and expensive, because the fruits

of this species are used in processing dye powder for food due to their non-toxic feature. On the other hand, choosing to find the mother trees which reach to meet the seed quality standards becomes scarce, so many mature trees grow and develop limitedly, productivity of fruit and seed yield is not high because the tree is mostly used for decorative purposes.

Unlike seedling tree, cuttings have the advantage of maintaining the same genetic quality as the orginal mother plant to make propagation, rapid multiplication in large numbers, new cuttings with high uniformity and early harvest (Tho V. Hoang, 2017; Tho V. Hoang, 2015; Tuan D. Pham, Tho V. Hoang, 2008). Therefore, research on the rooting ability of *G.jasminoides* species by cuttings method is very necessary, as well as it has scientific and practical meaning. The success of this research contributes to the provision of information and scientific basis for the clonal propagation, planting and development. This article introduces some research results for clonal propagation for *G.jasminoides* species that have achieved by cuttings method.

2. RESEARCH METHODOLOGY

2.1. Materials

The cuttings used in this study were taken directly from the over shoots of healthy, mature, more fruit mother plants grown in the families garden in Xuan Mai town, Chuong My district, Hanoi city. The process of transporting shoots was kept under conditions of avoiding sun and keeping cool. The cuttings were handled in time at the end of Fall in 2017 and the data is collected in April 2018 at the Vietnam Forestry University's nursery.

2.2. Research methodology

The used hormones were IBA, NAA at different concentrations and TTG (1% IBA powder, commercially available) to test the type and concentration that stimulated rooting ability in cuttings process for *Gardeniajasminoides* species. The hormones used in this study were at concentrations ranging from 250 to 1500 ppm, treated by rapid- immersion in solution of stimulated and puting the powdered on the root cuttings and gave the results of 10 formulas in total, in which CT1, CT2, CT3 and CT4 using IBA according to concentrations are: 250, 500, 1000 and 1500 ppm; and CT5, CT6, CT7 and CT8 using NAA at 250, 500, 1000 and 1500 ppm respectively; and CT9 using powdered stimulants, TTG (1% IBA) and CT10 (control formula, symbol is DC, do not use stimulants).

The experiments and controlled formulas were arranged in a randomized block completely with 3 replications, the number of samples for each experiments and controlled formula were 90 samples. After transplanting, the cuttings are shaded from the sun and kept moist regularly according to the usual technical procedures. The samples used in the experiment were taken from over shoots, 8 - 12 cm long, with 3 - 6 leaves were partially cut off the leaf area. To prevent pathogens during cuttings, substrates and cuttings are sterilized with Benlate by conventional methods.

Rooting ratio is calculated according to formula (1); The average number of roots per cuttings and the longest average root length per cuttings were calculated by formula (2); Statistical analysis was taken by the effect of the kinds of hormone and from concentration of IBA and NAA to rooting ratio tests using the Chi-squared standard (χ_n^2) and calculated according to formula (3).

$$Rooting\ ratio = \frac{Number\ of\ sample\ has\ roots}{Total\ of\ number\ sample\ of\ \text{experiment}}\ x\ 100\% \quad (1)$$

$$\overline{X} = \frac{1}{n}\sum_{i-1}^{n} X_i \quad (2); \qquad \chi_n^2 = \sum \frac{\left(f_t - f_l\right)^2}{f_l} \quad (3)$$

Statistical checking was performed according to standard of U in the exact standard of deviation to find the best formula which had the most effect on the rooting ability according to formula (4) if there were significant differences between two formulas. For rooting length, using the two-factor variance analysis was to test and compare between the two formulas which have the largest sample mean value for finding better formula according to t-test standard of student and formula (5) and (6) if there is a significant difference between the experimental formulas.

$$U = \frac{\overline{X} - \overline{X}}{S_n \sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}} \quad (4)\ ; \qquad t = \frac{\overline{X_i} - \overline{X_j}}{s_n \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \quad (5)\ ; \quad \text{Therein, } S_n = \sqrt{\frac{V_n}{n-a}} \quad (6)$$

Rooting index (RI) is a synthetic indicator reflecting the rooting quality system of the cuttings, and are calculated by equal multiplication between the average number of roots per cuttings and the average rooting length per cuttings such as according to formula:

$$RI = N \times L \qquad (7)$$

While RI is rooting index, N is number rooting average per formula; and L is length of rooting average per formula.

Collected data as processed individually using the statistical method used in forestry on the Excel 5.0 and SPSS application software.

3. RESULTS AND DISCUSSION

3.1. Effect types of hormones on rooting ability of cuttings

In this study, the used hormones (IBA and NAA) had the same concentration, TTG alone used as comparator. Results of the research about the effect of hormones on rooting ability during cuttings are summarized in table 1.

Table 1. Effect types of hormones on rooting rate of cuttings

Formula	Number of sample	Rooting ratio (%)	Total roots number	Num of roots average percuttings	Length of roots average percuttings (cm)	RI
IBA	90	**83.3**	2244	29.9	3.3	**98.7**
NAA	90	73.3	1112	16.8	**3.5**	58.8
TTG	90	66.7	849	14.9	3.3	49.2
DC	90	50.0	249	5.9	**3.9**	23.0
$\chi_n^2 = 36.8$						

Data in table 1 and Figure 1 show that the non-hormonal controlled formula only had the lowest rooting rate (50.0%), when using different hormones gave the different results in the setting. By using IBA, the rate of root cuttings, total root count and rooting index were 83.3%. 2244 and 98.7 out of IBA and TTG were 1.13 and 1.24 times; and 2.01 and 2.64 times; and 1.67 and 2.00 respectively for the same indicator (Figure 1).

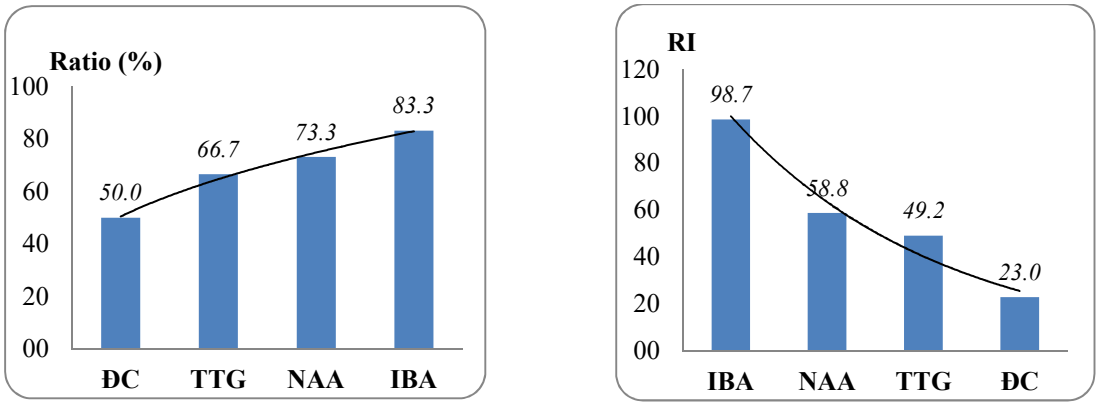

Figure 1. Ratio of rooting (left) and rooting index (right) by different types of hormones

However, data in table 1 also showed that the average root length per cuttings using NAA was 3.5 cm, which was 1.06 times greater than IBA and TTG using the same time and the same comparative criteria. Thus, although the usage of IBA did not yield large root length values, the average number of root per cuttings was highest and the rooting index was superior or the root quality was better. This means that the cuttings are more likely to survive, grow and develop more favorably at the same indicator, the same time and condition.

The results of statistical tests using the Chi-squared standard (χ^2_n) show that, χ^2_n (36.80) > $\chi^2_{05(k)}$ (5.99), (36.80) > (5.99), this confirms that the usage of different hormones has a significant effect on the ratio of rooting during the cuttings, in which IBA has more pronounced effect. In addition, the figures and graphs shown in Figure 1 also show that, the rooting ratio of *G.jasminoides* in the cuttings increased from DC, TTG, NAA to IBA, while rooting index tended to decrease from IBA, NAA, TTG to DC.

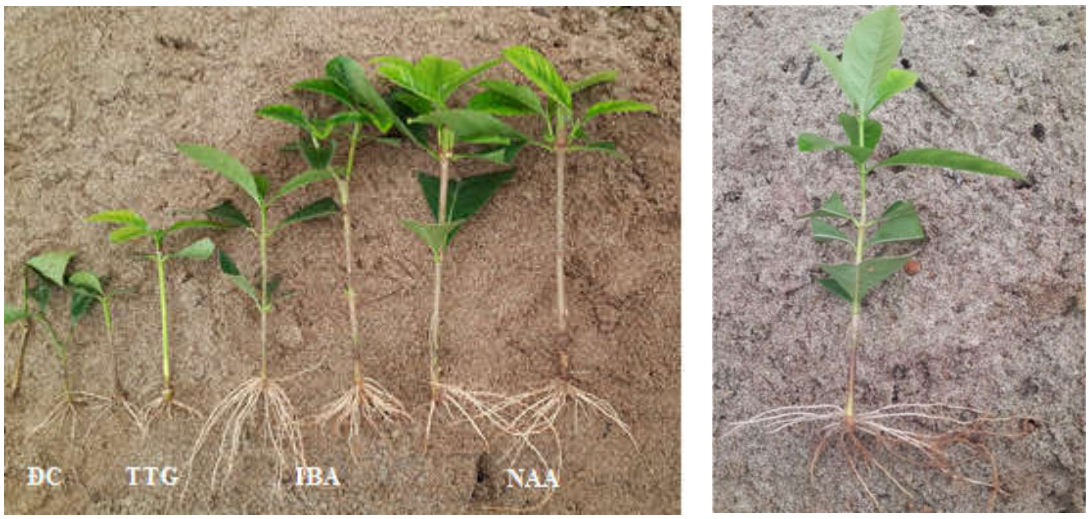

Figure 2. Rooting ability of different types of hormones

Data in table 1 and figure 1 also shows that, cuttings of *G. jasminoides* do not use stimulants to root out as control formula (DC) also having the results that reached a significant rooting rate of 50%. So, although *G. jasminoides* is a wooden tree species that is often more difficult to propagate by cuttings, the results of this study opened the possibility of clonal propagation for this species even in the absence of rootings stimulant (Figure 2). In other words, it is possible to propagate on *G. jasminoides* by cuttings method without using root stimulators, but to achieve better results it is necessary to use a larger number of cuttings than usually. Of course, the results of this study are only preliminary, so more deep researches are needed to obtain more accurate results.

3.2. Effects of IBA and NAA concentrations to rooting ability

**** Effect of IBA concentration to rooting ability of cuttings***

The information, characteristics and techniques for clonal propagation of *G. jasminoides* is limited and less well-known. Propagation and planting are mainly proceeded by seedlings from seeds with the primary purpose of making bonsai trees in many households, but not focus on the value as a pharmaceutical material of this plant. Moreover, this was the first study on clonal propagation for *G. jasminoides* by cuttings method, so finding types of hormones and appropriate concentration is essential, in order to increase ratio of rootings, root system quality and complete cuttings trees before they are ready to plant. The results of the study on the effect of type and concentration of hormones on rootings ability during cuttings are summarized in table 2.

Table 2. Effect of types and concentration of hormones on the rooting ratio

Formula	Rooting ratio (%)		Average number of roots per cuttings		Average roots length per cuttings (cm)		Rooting Index (RI)	
	$\bar{X}$	CV,%	$\bar{X}$	CV,%	$\bar{X}$	CV,%	$\bar{X}$	CV,%
CT1	66.7	26.0	10.1	49.1	3.8	37.7	36.9	23.5
CT2	80.0	28.9	10.2	39.4	3.6	37.3	36.6	7.8
CT3	76.7	26.5	18.7	48.3	3.8	38.4	71.8	10.3
CT4	83.4	13.8	29.9	53.5	3.3	38.2	97.9	4.7
	$\chi^2_n = 7.83$			$F_A = 9.63$			$F_A = 10.51$	
CT5	80.0	19.1	9.3	59.7	3.5	38.2	33.2	5.4
CT6	76.7	19.0	12.4	49.8	3.3	33.9	40.4	2.6
CT7	66.7	21.8	11.7	57.2	2.6	37.8	29.8	17.8
CT8	73.3	15.7	16.8	58.2	3.5	30.3	59.7	10.2
	$\chi^2_n = 4.57$			$F_A = 32.06$			$F_A = 18.44$	
CT9	66.7	30.5	14.9	65.1	3.3	44.3	49.7	5.2
DC	50.0	17.7	5.9	42.0	3.8	51.8	22.5	12.7

Data in table 2 shows that with the same hormone but using different concentrations took the results of rooting ratio, average number of roots per cuttings, average root length per cuttings and rooting index were very different. Accordingly, IBA at concentrations of 1500 ppm (CT4) had the rate of rooting (83.4%); average roots number per cuttings (29.9 roots per cuttings); and the rooting index (97.9) exceeds the formula; CT3; CT2; CT1; and DC is 1.09; 1.04; 1.25; and 1.67 times; 1.60; 2.93; 2.96; and 5.07 times; and exceeded 1.36; 2.67; 2.65; and 4.35 times respectively under the same tested conditions and time.

Thus, in this study, IBA at concentrations of 1500 ppm (CT4) gave the highest rooting rate (83.4%) compared to the other treatments (CT3, CT2 and CT1) with lower concentration (1000, 500 and 250 ppm respectively). Apparently, with the same hormone (IBA) but using the appropriate concentration, such as IBA at concentration 1500 ppm (CT4 formula) promotes better rooting ratio advantage (Table 2 and Figure 3).

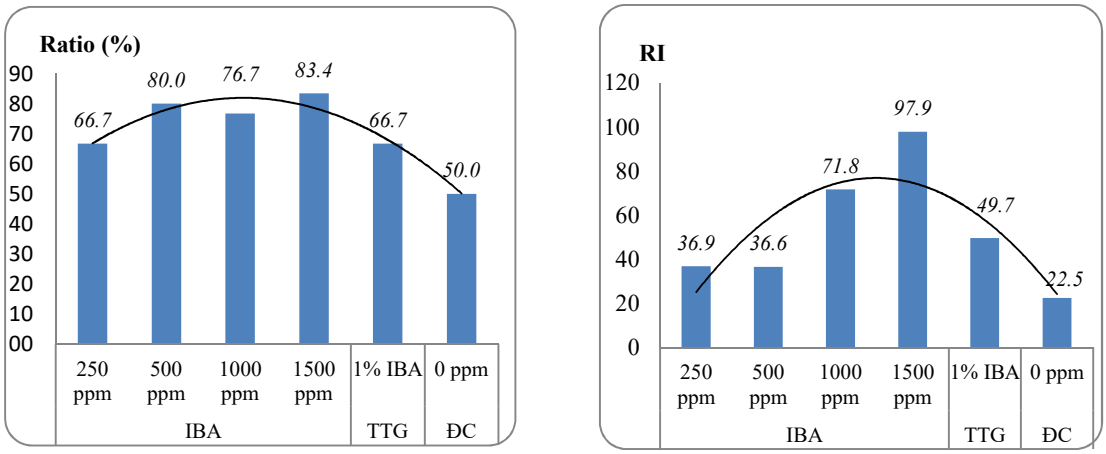

Figure 3. Rate of rooting (left) and rooting index (right) of IBA concentration

The results of testing the effect of IBA at concentration on rooting ability, average number of roots per cuttings and rooting index by Chi- squared standard χ^2_n and standard F showed that, χ^2_n (7.83) $> \chi^2_{05(k)}$ (7.81); F_A

(6.93) > F_{05} (4.96) và F_A (10.51) > F_{05} (3.10) (Table 2). Thus, it can be asserted that IBA concentration has a significant effect on rooting ratio, average number of roots per cuttings, and rooting index, while using IBA at concentration 1500 ppm has more effect.

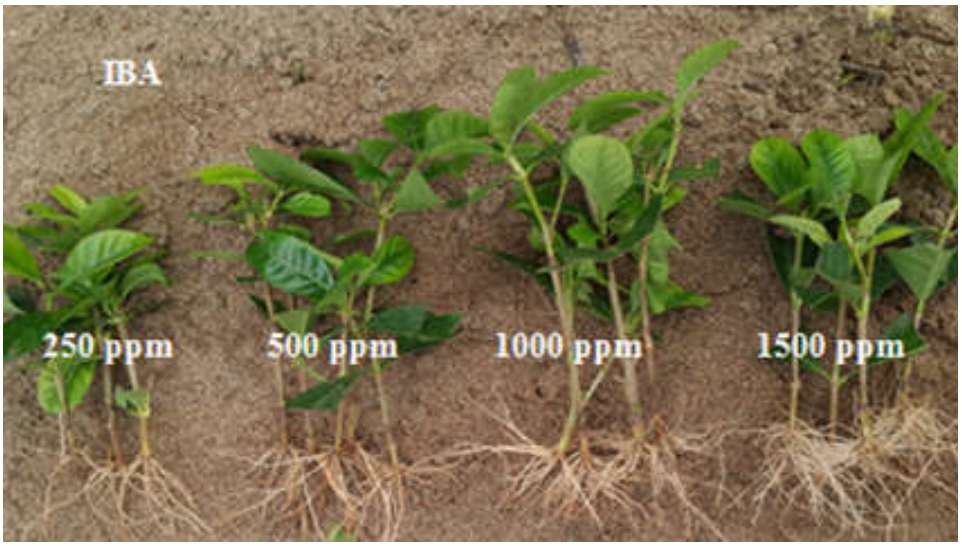

Figure 4. Rootins ability of *Gardenia jasminoides* acording to IBA concentration

On the other hand, the image (Figure 4) shows that the original premise of using IBA at low concentrations (250 ppm) such as CT1 formula had low rooted ratio, doubling the concentration of IBA (500 ppm) such as CT2, and continue increasing to higher concentration (1500 ppm) (6 times compared to the initial concentration used), the results is achieved from the highest rooting ratio (83.4%); the largest rooting index (97.9); and the quality of roots system of cuttings are also better. The question raised in this study, if we continue increasing the concentration of using IBA, for example 2000 ppm or higher, can the achieved result is in higher rootings ratio? Answering this question might be a hint for future studies with larger concentrations, which can determine the upper limit or maximum threshold for IBA concentration during cuttings for *Gardenia jasminoides*.

In summary, in this study, the use of IBA at concentration 1500 ppm was achieved the highest rooting ratio, average roots per cuttings and rooting index compared using IBA at the other concentrations under the same conditions and time. However this is only the initial results, so further research is needed with wider band of concentration to obtain more accurate results, as well as to identify the upper limit of the concentration IBA in clonal propagation of *Gardenia jasminoides*.

* *Effect of NAA concentration on rooting ratio ability of cuttings*

Data in Table 2 shows that formulas: CT5; CT6; CT7; and CT8 using NAA with a corresponding concentration of 250; 500; 1000; and 1500 ppm achieved the rooting ratio was 80.0; 76.7; 66.7 and 73.3% respectively; and the average number of roots per cuttings and rooting index was 9.3; 12.4; 11.7; and 16.8 roots; and 33.2; 40.4; 29.8; and 59.7 respectively under the same conditions and comparative criteria. Thus, using NAA at concentration of 250 ppm (CT5) gave the highest rooting rate (80.0%), the remaining formulas inluding: CT6, CT7 and CT8 only achieved lower roots ratio. Thus, using the NAA concentration of 250 ppm (CT5) gave the highest rooting rate (80.0%), the remaining treatments (CT6, CT7 and CT8) achieved the lower roots.

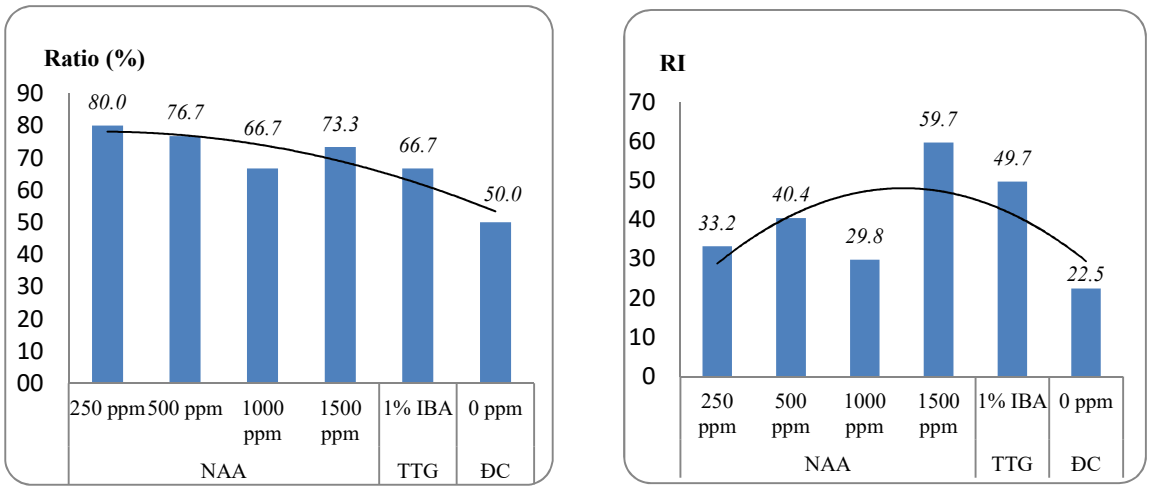

Figure 5. Rooting ratio (left) and RI (right) acording to the concentration of NAA

However, the average number of roots per cuttings and rooting index when using NAA at concentration 1500 ppm (CT8) reached the highest value of 16.8 roots per cuttings and 59.7, in comparison with other formulas: CT5; CT6; CT7; and DC are 1.81; 1.35; 1.44; and 2.85 times; and 1.80; 1.48; 2.00; and 2.65 times respectively. The results of testing the effect of NAA at concentration on rooting ratio ability by chi-aquared standard χ^2_n and F standard showed that, χ^2_n (4.57) < $\chi^2_{05(k)}$ (7.81). Thus, there is no clear difference in rooting ratio when using NAA at different concentrations. In addition, photographs and values on the histograms shown in Figure 5 and figure 6 on the rooting capacity of *Gardenia jasminoides,* with the concentration used NAA elucidated on the above discussed issues.

However, in order to determine the optimal NAA concentration that promotes rooting ratio during cuttings, intensive research is required, which is different from wider band of concentration for more accurate results.

Figure 6. Rooting ratio ability acording to different concentration of NAA

In summary, cuttings of *Gardenia jasminoides* used NAA at concentration of 250 ppm reached the highest rooting rate (80.0%), while at concentration 1500 ppm the results were obtained with the average number of roots per cuttings and the highest rooting index compared to other concentrations under the same condition and time. On the other hand, the results of this study suggested that more in-deep studies with wider-band concentrations are needed to obtain more accurate results, as well as to identify the upper limit when used

hormone of NAA in the cuttings for *Gardenia jasminoides*.

In the cuttings of wooden trees, high rooting rate is required, but it is important to produce complete cutting trees with good growth and development, meeting the requirements of the criteria for gardening to plant. Therefore, the study of the effect of hormones on the rooting ability of cuttings in the nursery stage is necessary, highly practical significance in clonal propagation for Gardenia jasminoides, and all of it will be presented in detail of this article below.

3.3. Effects of types hormone and concentration on the ability sprouting shoots

In this study, the effects of IBA and NAA and their concentration on the ability sprouting shoots of *Gardenia jasminoides* during cuttings are necessary, practical significance. The results are summarized in table 3.

Table 3. The ability sprouting shoots of different experemental formulas

Formula	Total number of shoots	Average number of shoots per cuttings		Average shoots length per cuttings (cm)	
		$\overline{X}$	CV,%	$\overline{X}$	CV,%
CT1	103	1.80	33.6	0.97	63.5
CT2	129	1.80	36.2	0.97	66.6
CT3	117	1.80	41.7	1.13	68.0
CT4	120	1.73	49.0	0.93	51.7
	$F_A = 191.24$			$F_A = 533.82$	
CT5	123	1.87	47.0	1.03	49.2
CT6	126	1.90	47.5	1.10	51.0
CT7	102	1.80	43.2	0.97	44.2
CT8	117	1.80	33.1	1.03	45.2
	$F_A = 193.17$			$F_A = 480.20$	
CT9	105	1.77	35.9	1.00	45.2
ĐC	75	1.80	31.6	0.90	44.2

The data in table 3 and figure 7 showed, different types of hormones and concentrations obtained results in process of cuttings with a total number of sprouting shoots are relatively different. Accordingly, using IBA and NAA at concentrations of 500 ppm (CT2 and CT6) obtained results with total sprouting shoots reached the value which are 129 and 126 shoots, significantly higher than CT1; CT3; CT4; and DC; and exceeding CT5; CT7; CT8; and DC are 1.25; 1.10; 1.08; and 1.72 times; and 1.02; 1.24; 1.08; and 1.68 times respectively, under the same conditions and comparative criteria.

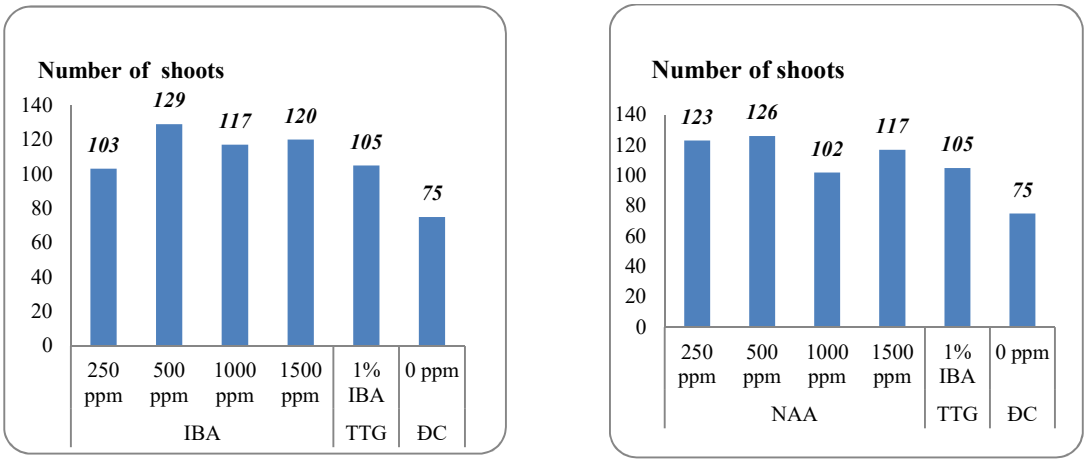

Figure 7. Total number of sprouting shoots of of *Gardenia jasminoides* turned on according to used IBA (left) and NAA (right)

Remarkable in this study, using IBA at high concentrations (1500 ppm), such as CT4, obtained results with the highest rooting rate of 83.4%; the rooting index was 97.9, exceeding the other formulas under the same conditions, while using IBA at lower concentrations (500 ppm) such as CT2 formula obtained more sprouting shoots.

In contrast, when using at low concentrations (250 and 500 ppm), NAA proved to be more favorable in promoting for *Gardenia jasminoides* rooting and sprouting shoots also more turned on under the same time and conditions (Figure 8).

Figure 8. Ability of sprouting shoots acording to used IBA (left) and NAA (Right)

Thus, in this study using IBA at high concentrations (1500 ppm) obtained results in high rooting ratio and reversely, when using NAA at low concentrations (250 and 500 ppm) promoted for *Gardenia jasminoides* rooting more and sprouting shoots more turned on under the same time and conditions.

Using IBA and NAA at a concentration of 500 ppm, which can be able to promote for *Gardenia jasminoides* sprouting shoots achieved highest value (129 and 126 shoots), while other using of high or low concentration are not obtained such high results.

4. CONCLUSIONS

From the results obtained at the above sections, it may be preliminary to give some conclusions:

1. Use type hormones and different concentrations obtained results with rooting ratio; average roots per cuttings; average root length per cuttings and rooting index; total number of shoots; average shoots per cuttings; and average shoots length per cuttings are very different, therein using IBA achieved higher results.

2. Use IBA at concentration of 1500 ppm obtained results with the highest rooting rate (83.4%), the average number of roots per cuttings was 29.9 roots, average root length per cuttings was 3.3 cm, and the achieved rooting index was 97.9.

3. Use NAA at concentrations of 250 and 500 ppm (CT5 and CT6) obtained results with the highest rooting ratio and sproutings shooting in total (80.0% and 126 shoots respectively), while at higher concentration (1500 ppm) with obtatined the results that reached the highest value with the average number of roots per cuttings, and rooting index are 16.9 and 59.7 respectively.

4. Use IBA (CT2) and NAA (CT6) at concentrations of 500 ppm in order to propagate *Gardenia jasminoides* by cuttings were able to promote sprouting shooting with highest achieved values were 129 and 126 respectively.

This is only the initial results, so further research is needed with wider band of concentration to obtain more accurate results, as well as to identify the upper limit of the concentration IBA and NAA in clonal propagation of *Gardenia jasminoides*.

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NGHIÊN CỨU KHẢ NĂNG RA RỄ CỦA DÀNH DÀNH (*Gardenia jasminoides)* BẰNG PHƯƠNG PHÁP GIÂM HOM

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[1]Trường Đại học Lâm nghiệp

TÓM TẮT

Bài viết giới thiệu kết quả nghiên cứu khả năng ra rễ của Dành dành (*Gardenia jasminoides*) bằng phương pháp giâm hom cho thấy, sử dụng loại hormone và nồng độ khác nhau thu được kết quả với tỷ lệ ra rễ; số rễ trung bình/hom; chiều dài rễ trung bình/hom và chỉ số ra rễ; tổng số chồi; số chồi trung bình/hom; và chiều dài chồi trung bình/hom là rất khác nhau, trong đó sử dụng IBA cho kết quả cao hơn. Sử dụng IBA nồng độ 1500 ppm (CT4) cho tỷ lệ ra rễ đạt trị số cao nhất (83,4%), số rễ trung bình là 29,9 rễ/hom, chiều dài rễ trung bình 3,3 cm/hom và chỉ số ra rễ đạt 97,9. Sử dụng NAA ở nồng độ 250 và 500 ppm (CT5 và CT6) cho tỷ lệ ra rễ và tổng số chồi đạt trị số cao nhất (80,0% và 126 chồi), trong khi ở nồng độ 1500 ppm thu được kết quả với số rễ trung bình/hom và chỉ số ra rễ đạt trị số cao nhất là 16,9 rễ/hom; và 59,7. Sử dụng IBA (CT2) và NAA (CT6) ở nồng độ 500 ppm, đều có khả năng xúc tiến hom Dành dành bật chồi với trị số cao nhất là 129 và 126 chồi tương ứng. Kết quả của nghiên cứu này là cơ sở cho các nghiên cứu tiếp theo để hoàn thiện qui trình nhân giống vô tính Dành dành cho gây trồng và phát triển.

Từ khóa: Giâm hom, IBA, NAA, nhân giống Dành dành, tỷ lệ ra rễ.

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